Title: **FLUOROMETRIC DETERMINATION OF CHLOROPHYLL A**

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## 1.0 OBJECTIVE

Chlorophyll *a* is used to estimate phototrophic biomass. The purpose of this method is to quantify chlorophyll *a* concentration from water samples. This method was adapted from Glover and Morris (1979).

## 2.0 HEALTH AND SAFETY

Personnel should wear lab coats and chemical resistant gloves.

## 3.0 PERSONNEL/TRAINING/RESPONSIBILITIES

Personnel should not perform this method until training by experienced individuals is complete.

## 4.0 REQUIRED AND RECOMMENDED MATERIALS

20 mL plastic scintillation vials
acetone
magnesium carbonate ($MgCO_3$)
deionized water
glass fiber filters (Type GF/F, 25 mm diameter)
filter apparatus
filter forceps
fluorometer (e.g. Sequoia-Turner Model 450)

disposable, borosilicate glass test tubes for fluorometer
Vortex mixer
1 and 10 mL pipettes/bulbs

## 5.0 PROCEDURE

### 5.1 Chlorophyll extraction

- Sample volume required will vary, depending on the chlorophyll concentration in the water. For PFU samples, 10 mL is typically adequate.
- Filter water sample onto glass fiber filter (Type GF/F, 25 mm diameter).
- Just before all sample passes the filter, rinse the column with two separate aliquots (1.0 mL each) deionized water. Continue vacuum until all liquid is gone.
- Release vacuum, disassemble filter tower apparatus, and remove filter with forceps.
- Place filter face up on bottom of scintillation vial, add 1 mL $MgCO_3$ and freeze until analysis.
- Samples should be kept in the dark for the rest of the procedure. To extract the samples, add 9 mL of acetone to each scintillation vial and shake well.
- Refrigerate samples overnight in the dark at 4 EC , shake the samples the next day, and refrigerate overnight again.
- The next day, bring the samples to room temperature and read on a fluorometer.

### 5.2 Fluorometric measurement

5.2.1 Chlorophyll *a*

- Before using the fluorometer for unknowns, a standard curve should be created with pure chlorophyll *a* extracts (available from Sigma).
- AZero@ each door opening of the fluorometer immediately prior to use, using a tube of 90% acetone.

- Decant the chlorophyll extract from the scintillation vial into the fluorometer test tube. Care should be taken not to transfer particulates from the filter into the tube.

- Wipe off the sides of the test tube with a Kim Wipe and place the test tube in the fluorometer.

- Record the fluorescence units, door setting and gain setting.

- Samples that are too concentrated may be diluted with 90% acetone.

- A new test tube should be used for each sample.

5.2.2 Phaeo-pigments

This extra step is performed to determine and correct for the concentration of phaeo-pigments (chlorophyll degradation products) in the samples. It is usually not necessary with the GF/F filtering technique, but is provided here for those using other collection methods.

- After the first reading is taken on the fluorometer, remove the tube and add 2 drops of 5% v/v hydrochloric acid.

- Mix contents of tube with a vortex mixer.

- Take a second reading 30-60 seconds later, after a stable value is reached.

**5.3 Calculations**

Chlorophyll *a* (µg/mL)= (door factor*(chlorophyll fluorescence reading-phaeo-pigment reading)*gain correction*acetone volume)/volume filtered*gain.

## 6.0 QUALITY CONTROL/QUALITY ASSURANCE

A minimum of three replicates per site or treatment is recommended. It is important that each sample is well-mixed prior to filtration and that the samples are kept in the dark after collection onto the filters.

## 7.0 REFERENCES

Glover H.E. and Morris I. 1979. Photosynthetic carboxylating enzymes in marine phytoplankton. Limnol Oceanogr 23:510-519